IN THE UNITED STATES DISTRICT COURT
FOR THE DISTRICT OF MARYLAND
(Southern Division)

Ex. 3

CLAUDIO DE SIMONE Plaintiff/Counterclaim Defendant, v. VSL PHARMACEUTICALS, INC., *et al.* Defendants/Counterclaim Plaintiffs, v. EXEGI PHARMA, LLC, *et al.*, Addt'l Counterclaim Defendants.	*	Case No. 8:15-cv-01356-TDC

DECLARATION OF CLAUDIO DE SIMONE

I, CLAUDIO DE SIMONE, make the following declaration under penalty of perjury pursuant to 28 U.S.C. § 1746:

1. I am over the age of 18 years and otherwise am competent to make this declaration.

2. I am the Plaintiff and Counterclaim Defendant in this action.

3. I make this declaration on the basis of personal knowledge and in opposition to the Motions for Preliminary Injunction and Order to Show Cause filed by Defendants/Counterclaims Plaintiffs VSL Pharmaceuticals, Inc. ("VSL Inc.") and Sigma-Tau Pharmaceuticals, Inc. ("STPI") in this action.

4. I hold a degree in Medicine and Surgery from the University of Rome, with specialization degrees in Gastroenterology and Immunology. Until my retirement, I was a

Professor of Infectious Diseases at the University of L'Aquila. I have authored over 200 published scientific articles, reviews, case reports and book chapters.

5. My research in the 1980s and 1990s focused on understanding the immunologic, metabolic, and cellular interaction between microorganisms and their human hosts.

6. Microorganisms administered through the mouth interact with the human host and its other microorganisms through genetic, immunologic, and enzymatic actions, as well as through direct cellular and extracellular matrix interactions.

7. In the mid 1990s, I invented an eight-strain, high-potency probiotic preparation with certain unique biochemical profiles. My intention was to ameliorate host diseases through various biochemical and immunological interactions between the host and the microorganisms. The preparation (the "De Simone Formulation") has been shown to be effective and safe for various serious conditions, such as Inflammatory Bowel Disease, radiation-induced diarrhea, and liver cirrhosis.

8. My understanding is that a contested issue in these pending proceedings is whether or not the De Simone Formulation contained in the product now sold under the brand name Visbiome is exclusively available from ExeGi Pharma, LLC, which is an Additional Counterclaim Defendant in this action. The De Simone Formulation is produced by Danisco USA, Inc. ("Danisco") under my direction. The De Simone Formulation can be manufactured only with my proprietary know-how, which is known only to me and Danisco.

9. In accordance with my instructions, and as authorized under particular contracts among myself, VSL Inc., and Danisco, I instructed Danisco to stop supplying the De Simone Formulation to VSL Inc. and STPI after January 31, 2016. Danisco has complied with that instruction. As a result, apart from the indeterminate amount of the Danisco-manufactured

product that STPI still may have in its inventory, the De Simone Formulation now is available in the United States only from ExeGi, to whom I have given exclusive rights to procure, market, and distribute the De Simone Formulation in the United States. Going forward, because VSL Inc. and STPI do not have my or Danisco's participation, it is impossible for them to manufacture, market, or sell a product that has the same biochemical properties as the De Simone Formulation.

10. Nevertheless, it has come to my attention that, in the U.S., VSL Inc. and STPI intend to launch or already have launched a purported copy of the De Simone Formulation labeled as VSL#3. In Holland and the United Kingdom, VSL Inc. has already launched this inauthentic copy product. I understand that this is the same product that VSL Inc. intends to launch in the U.S. after the exhaustion of their supply of VSL#3-branded product containing the actual De Simone Formulation produced by Danisco.

11. As demonstrated in this Declaration, VSL Inc.'s new product is not equivalent to the De Simone Formulation. Detailed below is an extensive review and analysis of the product, which is currently being sold under the "VSL#3" brand name in the United Kingdom and Holland. The analysis shows clear material distinctions between the De Simone Formulation and the new product.

First Testing Parameter: Live v. Dead Bacteria

12. The proportion of live to dead bacteria in a bacterial formulation is a key element and has a material affect on the formulation's efficacy. On the boxes of the original De Simone Formulation and VSL Inc.'s new formulation it is written: "Live freeze-dried bacteria. 450 billion bacteria per sachet." Live cells in probiotic products will inevitably lose viability, and the actual products will contain varying populations of dead cells. In practice, it is not possible to

feed only live bacteria to a subject. Since the dead bacteria affect the responses of the individual, controlling the quality of the product just by counting the live bacteria is a major mistake. Assessing the percentage of live bacteria and dead bacteria in a single sachet is an important parameter since: (a) the capability of the product to colonize the gut properly is related to the amount of live bacteria per sachet; (b) the quantity of dead bacteria is a good predictor of the quality of the product and its stability, and (c) dead bacteria are not an inert material.

13. Owing to the importance of the proportions of live and dead bacteria, the first parameter that we checked in determining whether VSL Inc.'s new product is the same as the De Simone Formulation was the percentage of live bacteria versus dead bacteria in a single sachet of each product. Testing of these proportions in each product was conducted by the laboratory Biovisible BV, located in Groningen, Holland. The Biovisible laboratory specializes in testing the quality of probiotic formulations.

14. The live/dead status of bacteria in the two "VSL#3" labeled products (one containing the real De Simone Formulation, produced by Danisco prior to February 1, 2016 and the second containing the new formulation, produced by a different supplier) was assessed using a mixture of SYTO® 13 green fluorescent nucleic acid stain and the red fluorescent nucleic acid stain, Propidium Iodide ("PI") (Life Technologies, Europe). These stains differ both in their spectral characteristics and in their ability to penetrate healthy bacterial cells. When used alone, the SYTO® 13 stain labels bacteria with both intact and damaged membranes. In contrast, PI penetrates only bacteria with damaged membranes, competing with the SYTO® 13 stain for nucleic acid binding sites when both dyes are present. When mixed in correct proportions, SYTO® 13 stain and PI produce green fluorescent staining of bacteria with intact cell membranes (*i.e.*, live bacteria) and red fluorescent staining of bacteria with damaged membranes

(*i.e.*, dead bacteria). The background remains virtually non-fluorescent. The standard live/dead viability assay (Life Technologies, Europe) uses SYTO® 9 instead of SYTO® 13. In this case, SYTO® 13 was used as an alternative since SYTO® 13 proved to have less background in the analysis.

15. Regarding the material and methods of the Biovisible analysis, 100 mg of each product was dissolved in 1 ml H20. Then, 2 µl of the probiotic mix was added to 100 µl of staining solution containing 10 µM PI and 8 µM SYTO® 13 and stored in the dark at room temperature for 15 minutes. After the incubation, the solution was diluted in 1 ml H20 and cells were collected on a 0.2-mm-pore-size Isopore polycarbonate membrane filter (Millipore Corporation) by filtration and washed with 4 ml H20. Filters were mounted on microscope slides with BacLight™ mounting oil (Life Technologies, Europe), and cells were inspected visually using an Olympus BX60 epifluorescence microscope using a FITC or Cy3-specific filter.

16. The results of the Biovisible analysis, showing the percentage of live and dead cells, are reported in Table 1. Figure 1 is a graphical depiction of the analysis.

Table 1. Percentage of live and dead cells from different "VSL#3" labeled batches

Measurements	507132* live (%)	507132* dead (%)	TM091** live(%)	TM091** dead(%)
1	40.0	60.0	69.1	30.9
2	46.2	53.8	59.7	40.3
3	38.5	61.5	64.7	35.3
4	40.2	59.8	67.0	33.0
5	47.2	52.8	58.2	41.8
6	42.0	58.0	78.6	21.4
7	47.3	52.7	71.9	28.1
8	45.7	54.3	62.0	38.0
9	49.1	50.9	62.1	37.9
10	48.3	51.7	57.6	42.4

AVG	44.46***	55.54****	65.08***	34.92****
SD	3.88	3.88	6.66	6.66

*) VSL#3 labeled lot 507132 ("new formulation")
**) VSL#3 labeled lot TM091 ("original" De Simone Formulation)
***) $p<0.0001$
****) $p<0.0001$

Statistical analysis of data was performed using one-way ANOVA followed by Bonferroni posttests (Prism 5.0 GraphPad Software, San Diego, CA). The significance level was set at $p<0.001$.

Figure 1. Original De Simone Formulation - VSL#3 TM091 cells stained with SYTO® 13 and PI. 1000x magnification.

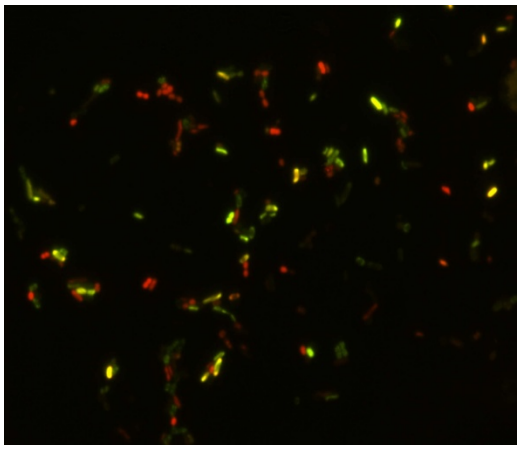
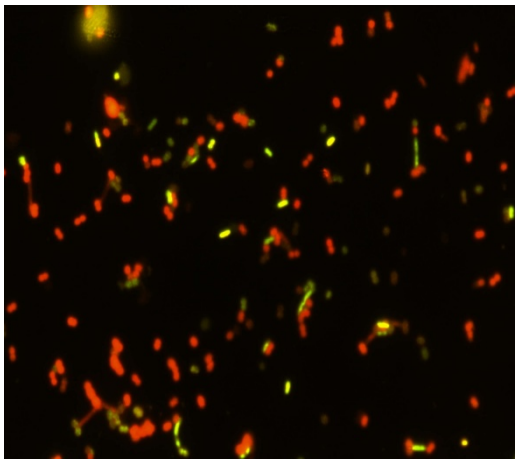


Figure 2. "New Formulation" VSL#3 507132 cells stained with SYTO® 13 and PI. 1000x magnification.



17. Since on both boxes of VSL#3-branded product it is written "Live freeze-dried bacteria. 450 billion bacteria per sachet," we must assume that each sachet contains 450 billion bacteria. Accordingly, the original De Simone Formulation-based product contains 241 billion (34.92%) of dead bacteria in addition to the 450 billion live bacteria (65.08%). The "new product" containing 450 billion of live bacteria (44.46%) also contains 562 billion dead bacteria (55.54%). The contents of the two sachets are therefore significantly different ($p<0.0001$).

18. To summarize, the live/dead bacteria for the single sachet is: VSL#3 TM091 (Original De Simone Formulation, Italy) = 691 billion (450 billion "live" + 241 billion "dead"), VSL#3 507132 ("new product", UK) = 1,012 billion (450 billion "live" + 562 billion "dead").

19. It is a common practice during the manufacturing process to "overfill" the sachet with bacteria. In other words, to maintain the target number of 450 billion live bacteria per sachet at the end of the expiry date of the product, the sachet is "overfilled" with more bacteria. Because of this practice, the number of dead bacteria ingested by a patient using this new product would be even larger than what is shown in the above analysis. In the case of a 700 billion "overfilling" (but the number can be easily 800 billion or more) by the manufacturer of the new product in order to maintain the target of 450 billion live bacteria at the end of the expiration period of the product, the amount of dead bacteria ingested by a patient is 3,946 billion per day in the event that he takes four sachets per day. The number of the dead bacteria ingested will increase proportionally to the increased "overfilling" and to the speed at which the live bacteria during the time span occurring between the manufacturing and expiry date of the product will become dead.

20. The dead bacteria cannot be considered a biologically inert material since they act as biological response modifiers. The single sachet of the "new product" has 233% more dead bacteria compared to the "original" product and this may affect significantly the response and health status of the person ingesting it.

Second Testing Parameter: Actual Number of Live Bacteria

21. Live cells in probiotic products inevitably lose their viability. The second parameter that we checked was the actual number of live bacteria by species in a single packet. The original De Simone Formulation product, having a 24-months expiry period ending in October 2017, was produced in October 2015, and at the moment of the testing, February 2016, was thus four months old. The new product, having an expiry date of July 2017, was estimated to have been manufactured in July 2015, and at the moment of the testing, February 2016, would have been thus seven months old. Both products claim to contain "live freeze-dried bacteria" with "450 billion bacteria per sachet."

22. The tests were performed at the University of Bologna, Italy, Department of Pharmacy and Biotechnology (Prof P. Brigidi, Full Professor, Delegate for European Research). For each sachet sample, 0.5g of granulated powder was taken and suspended in 50 ml of saline. From such stock, serial dilutions were performed in saline and dilutions -8, -9 and -10 were plated on the media MRS (Man, Rogosa and Sharpe broth), MRS pH 5.4, M17 and TOS+MUP.

23. The plates were then incubated under the following conditions:

- MRS in anaerobiosis at 37°C for 72 hours;
- MRS pH 5.4 in anaerobiosis at 37°C for 72 hours;
- TOS+MUP in anaerobiosis at 37°C for 72 hours and then for another 72 hours; and
- M17 in aerobiosis at 37°C for 48 hours.

24. With the method applied for a differential count of Bifidobacterium, Lactobacillus, and Streptococcus bacteria, the following results were obtained with the different culture media selective for specific bacterial populations:

Bacteria	**De Simone Formulation (contained in VSL#3 Lot No. TM091)**	**"New" VSL#3 Formulation (contained in VSL#3 Lot No. 507132)**
M17 (Streptococcus)	$1.05x10^{11}$ CFU/g	$1.40x10^{9}$ CFU/g
MRS (Bifidobacterium and Lactobacillus)	$3.11x10^{11}$ CFU/g	$2.08x10^{10}$ CFU/g
MRS pH 5.4 (Lactobacillus)	$5.00x10^{10}$ CFU/g	$1.61x10^{10}$ CFU/g
TOS Propionate + MUP (Bifidobacterium)	$3.11x10^{10}$ CFU/g*	$<1.00x10^{8}$ CFU/g

*Less than 10^8 means that in the sachet are present Bifidobacteria in the range of 10^7 or even below.

25. The results demonstrate that Streptococcus thermophilus (the major component of the product) is 2 log (100 times) less in the new VSL#3 formulation compared to the original De Simone Formulation ($1.40x10^9$ CFU/g versus $1.05x10^{11}$ CFU/g). Bifidobacterium (another major component of the product) are more than 100 times (more than 2 log) less in the new formulation versus the original (less than $1.00x10^8$ CFU/g versus $3.11x10^{10}$ CFU/g). No significant differences were observed between the two products regarding the lactobacilli. The fact that the lactobacilli are the same in both products is an indicator that both products had been maintained under the same conditions of storage and handling before and during the tests.

26. According to the above results, the sachet of the new VSL#3 formulation compared to the original De Simone Formulation has a 100 times less "live" Streptococcus thermophilus and Bifidobacterium, which is a significant loss even if we accept that the new

product was 3 months older than the original product at the time of testing. In any case, the quantities of these bacteria in the new formulation were far below (100 times less) the declared 450 billion live bacteria per sachet. Considering that at the moment of testing (February 2016) the new product still had 17 months of shelf life before expiry (July 2017), its stability is seriously questionable. This substantial difference between the original and new formulations is material and is likely to impact the efficacy of the new formulation.

Third Testing Parameter: Cell Division and Apoptosis Analysis

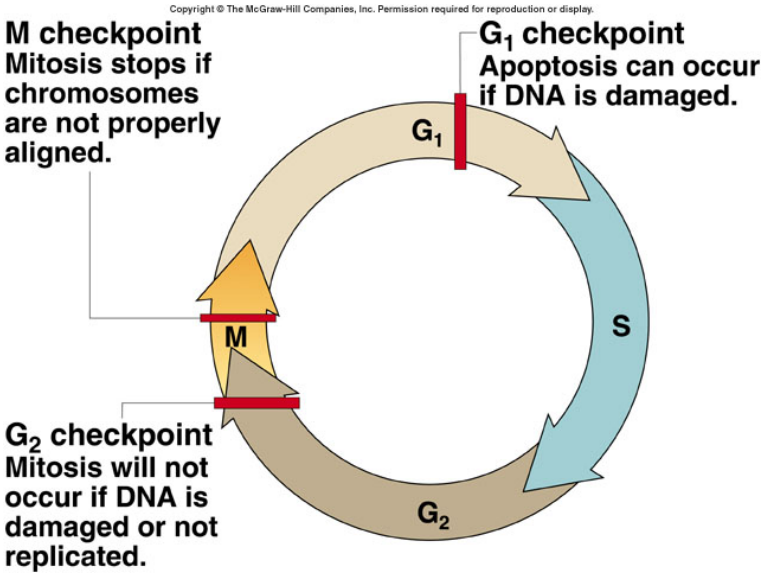
27. Our third testing parameter consisted of a cell cycle and apoptosis analysis conducted by Department of Life, Health & Environmental Sciences at the University of L'Aquila, Italy.

28. The cell cycle or cell division cycle is the series of events that take place in a cell leading to its division and duplication of its DNA (DNA replication) to produce two daughter cells. Regulation of the cell cycle involves processes crucial to the survival of a cell, including the detection and repair of genetic damage as well as the prevention of uncontrolled cell division. The molecular events that control the cell cycle are ordered and directional; that is, each process occurs in a sequential fashion and it is impossible to reverse the cycle. Cell cycle checkpoints are used by the cell for verification of necessary phase processes and repair of DNA damage. The cell cannot proceed to the next phase until checkpoint requirements have been met. Checkpoints typically consist of a network of regulatory proteins that monitor and dictate the progression of the cell through the different stages of the cell cycle.

29. There are several checkpoints to ensure that damaged or incomplete DNA is not passed on to daughter cells. Three main checkpoints exist: the G1/S checkpoint, the G2/M checkpoint, and the metaphase (mitotic) checkpoint. A dysregulation of the cell cycle

components may lead to tumor formation. When the cell is unable to pass the checkpoints, it undergoes apoptosis or programmed cell death. Between 50 and 70 billion cells die each day due to apoptosis in the average human adult. In contrast to necrosis, which is a form of traumatic cell death that results from acute cellular injury, apoptosis is a highly regulated and controlled process that confers advantages during an organism's lifecycle. In addition to its importance as a biological phenomenon, defective apoptotic processes have been implicated in a wide variety of diseases. As a matter of fact, a cell that lives past its "use-by-date" and is able to replicate and pass on any faulty machinery to its progeny has an increased chance of becoming cancerous or diseased. (*See* Figure 3 below.)

Figure 3. Cell Cycle and Apoptosis



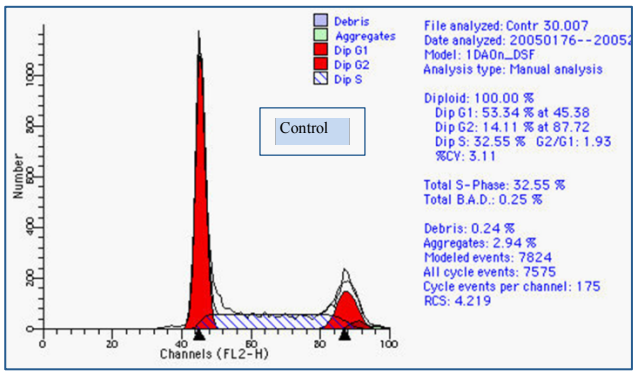
30. Probiotic bacteria are an accepted therapeutic strategy for chronic intestinal inflammation. One of the mechanisms of action by which probiotics exert their effects is the modulation of apoptosis in intestinal immune and/or epithelial cells. Therefore, knowledge of how the new VSL#3 formulation and the original De Simone Formulation modulate cell apoptosis is of major importance.

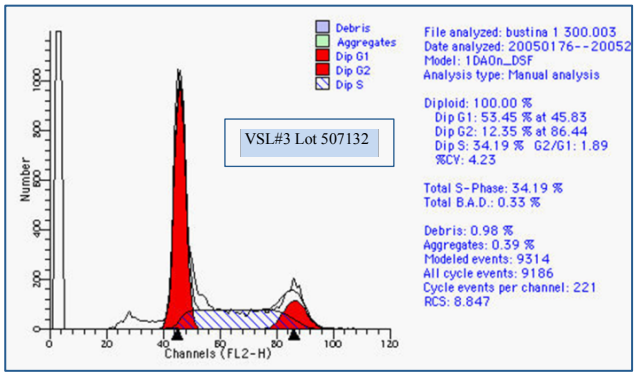
31. Testing of cell apoptosis was performed at the University of L'Aquila, Italy, Pathology Laboratory (Professor MG Cifone, Full Professor, Director of the Department Life, Health & Environmental Sciences). For cell cycle and apoptosis analysis, an immortalized tumor cell line (Jurkat) was used. The cells were treated with sachets of both the original De Simone Formulation and the new VSL#3 formulation (lot 507132 and lot TM091), for 18 hours, and then collected, dissociated, washed twice with ice-cold PBS and fixed in 70% ethanol at 4°C for 30 minutes. Subsequently, the fixed cells ($1x10^6$ cells/ml) were washed twice with ice-cold PBS and stained with solution containing 50 µg/mL propidium iodide, 0.1% Nonidet-P40, and RNase A ($6µg/1x10^6$ cell) for 30 minutes in the dark at 4°C.

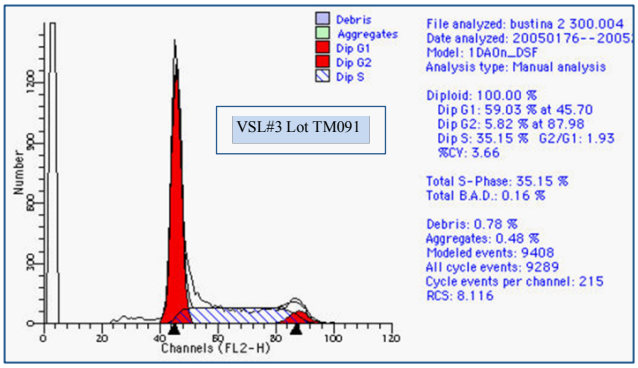
32. Cell cycle phase-distribution was analyzed by a flow cytometry system. Data from 10,000 events per sample were collected and analyzed using FACS Calibur (Becton Dickinson) instrumentation equipped with cell cycle analysis software (Modfit LT for Mac V3.0). Apoptotic cells were determined by their hypochromic subdiploid staining profiles and analyzed using Cell Quest software.

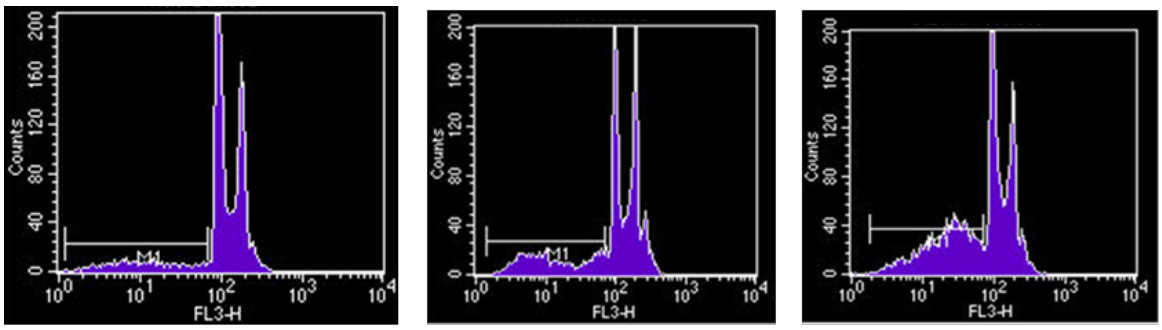
33. The results from one representative experiment from three are shown in Figure 4 below, and the mean values of three experiments are summarized in Table 2 below:

Figure 4. Cell Cycle profile and apoptosis analysis









Control

NEW
VSL#3
Lot 507132

ORIGINAL
VSL#3
Lot TM091

Table 2. Effects of probiotic blends on the cell cycles and programmed cell death

	Cell Cycle and apoptosis analysis (%)			
Sample	***G0/G1 phase***	***G2 phase***	***S phase***	***Apoptosis***
Control	55.14±2.55	11.33±3,92	33.52±1,37	10.79±3.45
"new" formulation	54.12±0.82	11.37±1,37	34.50±0,43	18.04±5.31 (^)
"original" Formulation	58.4±0.89 (*) (**)	7.58±2.58 (^) (**)	34.02±1,60	24.72±8.11 (^) (**)
n. 3 experiments in duplicate				
VSL#3 Lot 507132 (new product); VSL#3 Lot TM091 (original product)				
(*) p<0,05 vs control				
(^) p<0.01 vs control				
(**) p<0,01 vs "new product" (Lot 507132)				

34. A stop of the cycle in G1 means that the cell has "problems" (DNA errors) and accordingly it "stops" in G1 in an attempt to correct such DNA errors. If the cell can repair the damage, then the cycle goes on, and the cell does not die. On the other hand, if the cell fails to correct the DNA error, then it undergoes apoptosis. Basically, an arrest in G1 is propaedeutic to apoptosis, as shown in the Figure 3.

35. The original De Simone Formulation is statistically significantly different from the new VSL#3 product in its capability to arrest the cancerous cells in G0/G1 and in inducing the apoptotic death of cancer cells. In addition, consistent with the increased apoptosis, we observed a statistically significant reduced number of cancer cells treated with the original De Simone Formulation in the G2 phase, compared to the new VSL#3 formulation. This means that significantly fewer cancer cells will proliferate when treated with the original De Simone Formulation compared to the new formulation. Stated more simply, this test shows that the original De Simone Formulation is capable of increasing the death of cancer cells and to reduce their proliferation, while the new VSL#3 formulation failed to show any effect on the G2 phase arrest and it showed much lower efficacy in inducing apoptosis. This is an important parameter, since it is the final outcome of a very complex cascade of a number of immunological and biochemical pathways activated by the product. Notably, the many consumers who take this product for the dietary management of their inflammatory bowel disease are at high risk of colon cancer.

36. In January 2016, I was contacted by a patient from England (the "UK patient") who has been diagnosed with ulcerative colitis and who has been managing his conditions with use of VSL#3-branded probiotics along with other treatments prescribed by his doctor. Attached as Exhibit A is true and correct copy of the email I received from the UK patient, redacted to protect the patient's confidentiality.

37. The UK patient mentioned that his condition had been managed very well with the use of the original De Simone Formulation sold as "VSL#3" in past years (using 4 sachets/day) but recently he began to feel sick after he consumed a recently purchased box of "VSL#3" (lot number 507132 expiry date 07/2017) newly purchased from the pharmacy. He

went to the pharmacist where he made the purchase and learned that other consumers were bringing up similar complaints to the pharmacist and that the name of the strains listed on the box of the product were changed in the newly-purchased VSL#3.

38. The UK patient went on a search to purchase boxes of "VSL#3" reporting the original names of the strains (original De Simone Formulation) from a different source. He was successful in finding the original version and decided to resume taking of the product. After he started to take the original De Simone Formulation (lot LM537 expiry date 10/2017), his symptoms began to resolve.

39. Being puzzled, the UK patient questioned by e-mail the UK distributor of VSL#3 (Ferring UK) about the quality of the product, and after a few days received a written reply from Ferring UK. In the reply, Ferring UK sought to reassure him that nothing had changed with the product labeled "VSL#3", with the exception of the strains designation, which, according to Ferring UK, had been updated and "modernized." The UK patient was not satisfied by Ferring UK's reply, supported by the fact that to him also the taste and color of the new version of VSL#3 was different from the original version that he had come to reply upon.

40. The patient wrote again to Ferring UK inquiring if the manufacturer of the product had changed. Ferring UK replied that it passed the question on to the manufacturer. Suspicious, the UK patient contacted me to learn why this "new VSL#3" made him very sick. This inquiry prompted me to investigate the existence of a "new VSL#3" product launched in the UK, and to determine if this "new" formulation has the same properties as the original De Simone Formulation, which is the product manufactured by Danisco and studied and commercialized for more than 10 years.

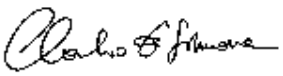
41. Consequently, boxes of VSL#3-branded product, distributed by Ferring UK, were purchased in the UK (lot number 507132, expiry date 07/2017; "new" formulation; the same lot the UK patient ingested) and were sent according to the manufacturer's handling instructions to three different centers (University of Bologna, University of l'Aquila, and Biovisible BV) for testing and comparison with the product distributed by Ferring in Italy (lot TM091; expiry date 10/2017); "original" formula).

42. The product in the box with batch number 507132 (new formulation), according to the declaration by Ferring on the box, contains strains genetically identical to that of the original De Simone Formulation (Lot number TM091) in the same number and ratio. Counting only the live bacteria and relying on a ratio number is not the proper way to assess the identity of a biological product like the De Simone Formulation.

43. Probiotic bacteria are living cell lines. They are sensitive to changes in growth conditions, purification processes, lyophilization, and blending and handling/storage conditions. These conditions and processes are difficult to characterize, quantify and reproduce by third parties. Even minor alterations in the production process may lead to significant changes in cell behavior and cause differences in bacterial structure, stability or other immunological and biochemical aspects of the end product. The results of the tests that were undertaken at my initiative, as reported in this declaration, confirm this reality of biologic product and that the new VSL#3 product is significantly different from the product that I have invented.

44. I declare under the penalty of perjury that the foregoing is true and correct.

Executed on: March 23, 2016



Claudio De Simone